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7	612519	aliphatic or aromatic	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/01/03 12:20
13	33	dipeptidylpeptidase and (aliphatic or aromatic)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/01/03 12:20
19	31681	(n-terminal) or (n-terminus)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/01/03 12:20
25	23	((n-terminal) or (n-terminus)) and (dipeptidylpeptidase and (aliphatic or aromatic))	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/01/03 12:21

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L2           3 L1 (10A) (ALIPHATIC OR AROMATIC)

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Bugno; Marcin, Chapel Hill, NC, US
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IN Banbula Agnieszka; Bugno Marcin; Potempa Jan S; Travis James
PAF The University of Georgia Research Foundation, Inc, Athens, 30602-7411, US
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AI US 2001-8355 20011108
PRAI US 2000-246827P 20001108 (Provisional)
FI US 2002164759 20021107
DT Utility; Patent Application - First Publication
FS CHEMICAL
APPLICATION
GOVI (0002) The present invention was made with government support under Grant No. DE 09761, awarded by the National Institutes of Health. The Government may have certain rights in this invention.
CLMN 23
GI 9 Figure(s).
FIG. 1 depicts a plot of the absorbance at 280 nm (A) and amidolytic activity against Ala-Phe-pNA (*) for the purification of P. gingvivalis **dipeptidylpeptidase** (DPP-7) from the acetone precipitate of the P. gingivalis cell extract. The straight solid lines indicate gradients in the eluting composition.
FIG. 1(a) illustrates the separation of DPP-7 on hydroxyapatite (100 ml) equilibrated with 20 mM potassium phosphate buffer, pH 7.0, and using a potassium phosphate gradient from 20 mM to 300 mM.
FIG. 1(b) illustrates the separation of DPP-7 obtained from the previous step on Phenyl-Sepharose HP (25 ml) equilibrated with 50 mM potassium phosphate, 1M ammonium sulfate, pH 7.0, at a flow rate of 30 ml/hour, and using an ammonium sulfate gradient from 0.4M to 0 M.
FIG. 1(c) illustrates the separation of DPP-7 on a MonoS FPLC column using a sodium chloride gradient from 0M to 0.3M then from 0.3M to 1M.
FIG. 2 is a depiction of the SDS-PAGE of fractions obtained during the purification of P. gingivalis DPP-7 with Lane A representing molecular mass markers (phosphorylase B, 97 kDa; bovine serum albumin, 68 kDa; ovalbumin, 43 kDa; carbonic anhydrase, 30 kDa; soybean trypsin inhibitor, 20 kDa; alphalactalbumin, 14 kDa); Lane B representing acetone precipitate from Triton X-100 extract of P. gingivalis; Lane C representing hydroxyapatite column eluate; Lane D representing PhenylSepharose column eluate; and Lane E representing MonoS column eluate.
FIG. 3 depicts a plot of the DPP-7 activity against Ala-Phe-pNA vs. pH. Enzyme activity was tested on Ala-Phe-pNA substrate in different buffers including: HEPES (*); PIPES (*); potassium phosphate (*); Tris (composite-function); and MES (uptriangle-filled).
FIG. 4 depicts the coding sequence (SEQ ID NO:1) encoding P. gingivalis DPP-7 (SEQ ID NO:2). Sequences obtained from the Edman degradation of the trypsin fragmented DPP-7 polypeptide chain are underlined. The putative

active site serine residue is marked by the black background.

FIG. 5 is a listing of sequences comparing the C-terminal regions of the P. gingivalis DPP-7 (residues 664-695; SEQ ID NO:3) and S. aureus V8 endopeptidase (residues 704-863; SEQ ID NO:4). Common residues are indicated by the single letter amino acid in the line between the two sequences. The "+" symbol in the line between the two sequences indicates similar residues.

FIG. 6 depicts a multiple sequence alignment of P. gingivalis DPP-7 and its putative homologues. Sequences of DPP-7 related proteinases were obtained from the conceptual translation of the following ORFs retrieved from unfinished and finished genomes databases (available at www.tigr.org): S1-Shewanella putrefaciens gnl vert-bar TIGR-24 vert-bar sputre 6401 (SEQ ID NO:5); S2-Shewanella putrefaciens gnl vert-bar TIGR-24 vert-bar sputre 6410 (SEQ ID NO:6); X-Xylella fastidiosa gb vert-bar AE004008.1 vert-bar (SEQ ID NO:7); P1-Porphyromonas gingivalis gnl vert-bar TIGR vert-bar P. gingivalis_CPG.con (SEQ ID NO:8); P2-P. gingivalis DPP-7 gnl vert-bar TIGR vert-bar P. gingivalis CPG.con (SEQ ID NO:9). The sequences were subsequently aligned using the ClustalW multiple sequence alignment tool.

AB The present invention provides isolated polypeptides, **dipeptidylpeptidases**, active analogs, active fragments, or active modifications thereof, having amidolytic activity for cleavage of a peptide bond between the second and third amino acids from the N-terminal end of a target polypeptide, wherein the target polypeptide has an aliphatic or an aromatic residue as a substituent on the alpha -carbon atom of the second amino acid from the N-terminal end of the peptide. Isolated nucleic acids encoding **dipeptidylpeptidases** are also provided, as are methods of reducing growth of a bacterium by inhibiting a **dipeptidylpeptidase**.

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TI Novel isolated **dipeptidylpeptidase** useful for identifying inhibitor of the **dipeptidylpeptidase** for protecting an animal from periodontal disease caused by Porphyromonas gingivalis; recombinant enzyme protein production useful in disease therapy and drug screening
AU TRAVIS J; POTEMPA J S; BANBULA A; BUGNO M
PA UNIV GEORGIA RES FOUND INC
PI WO 2002038742 16 May 2002
AI WO 2000-US46782 8 Nov 2000
PRAI US 2000-246827 8 Nov 2000
DT Patent
LA English
OS WPI: 2002-490075 [52]
AB DERWENT ABSTRACT:
NOVELTY - An isolated **dipeptidylpeptidase** (I) or its active analog, having amidolytic activity for cleavage of a peptide bond between the second and third amino acids from the N-terminal end of a target polypeptide (TP), where TP has an aliphatic or an aromatic residue as a substituent on the alpha-carbon atom of the second amino acid from the N-terminal end of TP, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated polypeptide (II) comprising at least 40 % identity with a 712 residue amino acid sequence (S1), given in the specification; (2) an isolated nucleic acid (III) comprising a coding sequence encoding (I) or its active analog, active fragment, or active modification having amidolytic activity for cleavage of a peptide bond between the second and third amino acids from the N-terminal end of a target polypeptide, where the target polypeptide has an aliphatic or an aromatic residue as a substituent on the alpha-carbon atom of the second amino acid from the N-terminal end of the polypeptide; (3) an isolated

nucleic acid (IV) encoding (II); (4) an immunogenic composition (V) comprising (I) or its antigenic analog, antigenic fragment or antigenic modification having amidolytic activity for cleavage of a peptide bond present in a target polypeptide, where the peptide bond is located between the second and third amino acids from the N-terminal end of the target polypeptide, and the second amino acid from the **N-terminal** end of the polypeptide has an **aliphatic** or an aromatic residue as a substituent on the alpha-carbon atom; and (5) a composition (VI) comprising an inhibitor of (I).

WIDER DISCLOSURE - A coding region sharing a significant level of primary structure with the coding region present at S1.

BIOTECHNOLOGY - Preferred Polypeptide: (I) is a serine protease isolated from Porphyromonas gingivalis. (I) is encoded by a 2139 base pair sequence (S3), given in the specification. Preferred Nucleic Acid: A complement of (III) hybridizes to (S3) under hybridization conditions of 0.5 M phosphate buffer, pH 7.2, 7 % sodium dodecyl sulfate (SDS), at 65 degrees C, where at least 20 nucleotides of the complement hybridize. Preferred Composition: In (V), the second amino acid is selected from alanine, phenyl alanine, isoleucine, and leucine.

ACTIVITY - Antiinflammatory; Antibacterial. No biological data is given.

MECHANISM OF ACTION - Inhibitor of (I).

USE - (I) is useful for identifying an inhibitor of (I), or its active analog, active fragment, or active modification, by identifying a compound that inhibits the amidolytic activity of (I) by incubating (I) with the compound under conditions that promote amidolytic activity of (I), and determining if the amidolytic activity of (I) is inhibited relative to the amidolytic activity in the absence of the compound. (VI) is useful for reducing growth of a bacterium by inhibiting (I) or its active analog, active fragment, or active modification, by contacting (I) with (VI), and for protecting an animal from a periodontal disease caused by Porphyromonas gingivalis by administering (VI) to the animal, where the disease is gingivitis or periodontitis. (All claimed). (I) is useful for reducing growth of bacteria, preferably a bacterial pathogen that causes periodontal disease such as P. gingivalis in vitro or in vivo. (V) is useful for protecting an animal from a disease caused by P. gingivalis.

ADMINISTRATION - The inhibitor of (I) is administered by subgingival application or controlled release delivery. No dosage is given.

EXAMPLE - Porphyromonas gingivalis DPP-7 was purified from strain HG66. The cells were grown and protein concentration was determined. The localization of active enzyme was checked in bacterial cells that had been subjected to a previously described fractionation procedure. All fractions, as well as the full culture, culture medium, and full culture after sonication, were assayed for amidolytic activity against H-A-Fe-pNA. Enzyme purification was performed, and the cells were collected by centrifugation and resuspended in 50 mM potassium phosphate buffer. The outer membrane proteins were solubilized with 0.05 % Triton X-100. After 2 hours of gentle stirring, unbroken cells were removed by centrifugation. Proteins from the supernatant were precipitated with cold acetone collected by centrifugation, and redissolved in 50 mM potassium phosphate buffer. After extensive dialysis against the same buffer the sample was loaded onto a hydroxyapatite column previously equilibrated with 20 mM potassium phosphate. The column was then washed until the A280 fell to zero. Bound proteins were eluted with a potassium phosphate gradient and fractions were analyzed for amidolytic activity against H-A-P-pNA. The active fractions were saturated with 1 M ammonium sulfate and loaded onto a Phenyl-Sepharose HP column equilibrated with 50 mM potassium phosphate. The column was washed with two volumes of the equilibration buffer, followed by a wash with buffer containing 0.4 M ammonium sulfate, and developed with a descending gradient of ammonium sulfate from 0.4-0 M. Active fractions were pooled, extensively dialyzed

against 20 mM 2-morpholinoethanesulfonic acid (MES), pH 6.6 and applied onto a MonoS HR 5/5 fast pressure liquid chromatography (FPLC) column equilibrated with the same buffer. Bound proteins were eluted with a 0-300 mM NaCl gradient. A homogeneous preparation of active proteinase was obtained. Electrophoretic techniques were used to monitor enzyme purification and estimate the enzyme molecular mass. The purified **dipeptidylpeptidase** was subjected to in-gel tryptic digestion. Peptides were extracted and separated by microbore reverse-phase high pressure liquid chromatography (HPLC). Fractions absorbing at 210 nm were manually collected, and their masses were determined. Selected peptides were subjected to Edman degradation. The DPP-7 coding sequence was identified. An unfinished P. gingivalis W83 genome database, available from the institute for genomic research, was searched for the presence of nucleotide sequences corresponding to the amino-terminal and the internal DPP-7 amino acid sequences using the TBLASTN algorithm. (65 pages)

L7 ANSWER 3 OF 5 WPINDEX (C) 2003 THOMSON DERWENT
AN 2002-490075 [52] WPINDEX
DNC C2002-139156
TI Novel isolated **dipeptidylpeptidase** useful for identifying inhibitor of the **dipeptidylpeptidase** for protecting an animal from periodontal disease caused by Porphyromonas gingivalis.
DC B04 D16
IN BANBULA, A; BUGNO, M; POTEMPA, J S; TRAVIS, J
PA (UYGE-N) UNIV GEORGIA RES FOUND INC
CYC 98
PI WO 2002038742 A2 20020516 (200252)* EN 65p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 2002025954 A 20020521 (200260)
US 2002164759 A1 20021107 (200275)
ADT WO 2002038742 A2 WO 2001-US46782 20011108; AU 2002025954 A AU 2002-25954 20011108; US 2002164759 A1 Provisional US 2000-246827P 20001108, US 2001-8355 20011108
FDT AU 2002025954 A Based on WO 200238742
PRAI US 2000-246827P 20001108; US 2001-8355 20011108
AB WO 200238742 A UPAB: 20020815
NOVELTY - An isolated **dipeptidylpeptidase** (I) or its active analog, having amidolytic activity for cleavage of a peptide bond between the second and third amino acids from the N-terminal end of a target polypeptide (TP), where TP has an aliphatic or an aromatic residue as a substituent on the alpha -carbon atom of the second amino acid from the N-terminal end of TP, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) an isolated polypeptide (II) comprising at least 40 % identity with a 712 residue amino acid sequence (S1), given in the specification;

(2) an isolated nucleic acid (III) comprising a coding sequence encoding (I) or its active analog, active fragment, or active modification having amidolytic activity for cleavage of a peptide bond between the second and third amino acids from the N-terminal end of a target polypeptide, where the target polypeptide has an aliphatic or an aromatic residue as a substituent on the alpha -carbon atom of the second amino acid from the N-terminal end of the polypeptide;

(3) an isolated nucleic acid (IV) encoding (II);

(4) an immunogenic composition (V) comprising (I) or its antigenic analog, antigenic fragment or antigenic modification having amidolytic activity for cleavage of a peptide bond present in a target polypeptide,

where the peptide bond is located between the second and third amino acids from the N-terminal end of the target polypeptide, and the second amino acid from the **N-terminal** end of the polypeptide has an **aliphatic** or an aromatic residue as a substituent on the alpha -carbon atom; and

(5) a composition (VI) comprising an inhibitor of (I).

ACTIVITY - Antiinflammatory; Antibacterial.

No biological data is given.

MECHANISM OF ACTION - Inhibitor of (I).

USE - (I) is useful for identifying an inhibitor of (I), or its active analog, active fragment, or active modification, by identifying a compound that inhibits the amidolytic activity of (I) by incubating (I) with the compound under conditions that promote amidolytic activity of (I), and determining if the amidolytic activity of (I) is inhibited relative to the amidolytic activity in the absence of the compound. (VI) is useful for reducing growth of a bacterium by inhibiting (I) or its active analog, active fragment, or active modification, by contacting (I) with (VI), and for protecting an animal from a periodontal disease caused by Porphyromonas gingivalis by administering (VI) to the animal, where the disease is gingivitis or periodontitis. (All claimed). (I) is useful for reducing growth of bacteria, preferably a bacterial pathogen that causes periodontal disease such as P. gingivalis in vitro or in vivo. (V) is useful for protecting an animal from a disease caused by P. gingivalis. Dwg.0/6

L7 ANSWER 4 OF 5 USPATFULL
AN 2000:15472 USPATFULL
TI Methods of identifying agonists or antagonists of angiotensin IV
IN Harding, Joseph W., Pullman, WA, United States
Wright, John W., Pullman, WA, United States
PA Washington State University Research Foundation, Pullman, WA, United States (U.S. corporation)
PI US 6022696 20000208
AI US 1998-54308 19980402 (9)
RLI Division of Ser. No. US 360784
DT Utility
FS Granted
EXNAM Primary Examiner: Mertz, Prema; Assistant Examiner: Hamud, Fozia
LREP Christensen O'Connor Johnson & Kindness PLLC
CLMN Number of Claims: 7
ECL Exemplary Claim: 1
DRWN 28 Drawing Figure(s); 16 Drawing Page(s)
LN.CNT 4234
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A unique and novel angiotensin AT4 receptor and AIV ligand system for binding a small N-terminal hexapeptide fragment of Angiotensin II (referred to as AIV, with amino acid sequence Val.sub.1 -Tyr.sub.2 -Ile.sub.3 -His.sub.4 -Pro.sub.5 -Phe.sub.6 ; SEQ. ID. NO. 1) is disclosed. AIV ligand binds saturably, reversibly, specifically, and with high affinity to membrane AT4 receptors in a variety of tissues, including heart, lung, kidney, aorta, brain, liver, and uterus, from many animal species. The AT4 receptor is pharmacologically distinct from classic angiotensin receptors (AT1 or AT2). The system employs AIV or C-terminally truncated or extended AIV-like peptides (e.g., VYIHPFX; SEQ. ID. NO. 8) as the signaling agent, and the AT4 plasma membrane receptor as the detection mechanism. The angiotensin AT4 receptor and receptor fragments (including the receptor binding site domain) are capable of binding a VYIHPF (SEQ. ID. NO. 1) angiotensin AIV N-terminal peptide but not an angiotensin AII or AIII N-terminal peptide, i.e., DRVYIHPF (SEQ. ID. NO. 2) or RVYIHPF (SEQ. ID. NO. 3), respectively. Also disclosed are processes for isolating angiotensin AT4 receptor and AIV angioteninase, identifying angiotensin AIV agonists and antagonists,

and constructing diagnostic assays to specifically measure AIV and AI-specific angiotensinase in biological fluids.

L7 ANSWER 5 OF 5 USPATFULL
AN 1998:162647 USPATFULL
TI Angiotensin IV peptides and receptor
IN Harding, Joseph W., Pullman, WA, United States
Wright, John W., Pullman, WA, United States
PA Washington State University Research Foundation, Pullman, WA, United States (U.S. corporation)
PI US 5854388 19981229
WO 9400492 19940106
AI US 1994-360784 19941222 (8)
WO 1993-US6038 19930624
19941222 PCT 371 date
19941222 PCT 102(e) date
DT Utility
FS Granted
EXNAM Primary Examiner: Robinson, Douglas W.; Assistant Examiner: Harle, Jennifer
LREP Christensen O'Connor Johnson & Kindness PLLC
CLMN Number of Claims: 14
ECL Exemplary Claim: 1
DRWN 28 Drawing Figure(s); 16 Drawing Page(s)
LN.CNT 4073
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A unique and novel angiotensin AT4 receptor and AIV ligand system for binding a small N-terminal hexapeptide fragment of Angiotensin II (referred to as AIV, with amino acid sequence Val.sub.1 -Tyr.sub.2 -Ile.sub.3 -His.sub.4 -Pro.sub.5 -Phe.sub.6 ; SEQ. ID. NO. 1) is disclosed. AIV ligand binds saturably, reversibly, specifically, and with high affinity to membrane AT4 receptors in a variety of tissues, including heart, lung, kidney, aorta, brain, liver, and uterus, from many animal species. The AT4 receptor is pharmacologically distinct from classic angiotensin receptors (AT1 or AT2). The system employs AIV or C-terminally truncated or extended AIV-like peptides (e.g., VYIHPFX; SEQ. ID. NO. 8) as the signaling agent, and the AT4 plasma membrane receptor as the detection mechanism. The angiotensin AT4 receptor and receptor fragments (including the receptor binding site domain) are capable of binding a VYIHPF (SEQ. ID. NO. 1) angiotensin AIV N-terminal peptide but not an angiotensin AII or AIII N-terminal peptide, i.e., DRVYIHPF (SEQ. ID. NO. 2) or RVYIHPF (SEQ. ID. NO. 3), respectively. Also disclosed are processes for isolating angiotensin AT4 receptor and AIV angioteninase, identifying angiotensin AIV agonists and antagonists, and constructing diagnostic assays to specifically measure AIV and AI-specific angiotensinase in biological fluids.